REMARKS/ARGUMENTS

Applicants would like to thank Examiners Woitach and Ton for the very helpful interview on April 14, 2005. As suggested by the Examiners, Applicants have filed a Request for Continued Examination to provide the Examiners with sufficient time to consider the remaining issues.

As discussed at the interview, the application is based on the discovery that one can combine the techniques of conventional nuclear transfer with the technique of homologous recombination, which can be used to produce a genetically modified cell that can be used in the production of a genetically modified tissue or animal. Prior to this invention, those in the art were using random integration of a gene to express a protein in transgenic animals. This led to the possibility of inadvertent damage to the genome and unwanted side effects, and did not allow for targeted disruption of genes.

After entry of the amendment, claims 62-67, 70-73, 75- 90, 98-100, 102-127, and 131-133 remain pending. As requested by the Examiner, claims 91-97 and 128-130 have been canceled without prejudice. Claims 62, 73, 90, 105, and 131-133 have been amended. Claim 101 has also been canceled without prejudice.

Claim Rejections - 35 U.S.C § 112

35 USC § 112 1st Paragraph Rejections

In the Final Office Action mailed on January 24, 2005, the Examiner rejected the pending claims under 35 USC § 112, first paragraph. The Examiner rejected Claims 62-67, 70-73, 75-90, 98-127 and 131-133 as failing to comply with the enablement requirement on various grounds, which are each addressed below.

(i) Somatic Donor Cells

The Examiner has rejected Claims 62-67, 70-73, 75-90, 98-127 and 131-133 as failing to comply with the enablement requirement on the basis that the state of the art of nuclear transfer with respect to the specific donor cell to be used is unpredictable. In particular, the Examiner cites Oback (Cloning & Stem Cells (2002) 4(2): 147-168) to support the contention of unpredictability because Oback shows that in different animal species, different somatic cells

have been tested with varying results, and provides a summary of cloning efficiencies from various cell types in Table 1 of the article.

Applicants have amended the claims to recite "A method for producing a non-human transgenic animal, the method comprising: (a) modifying the nuclear genome of a somatic cell with a normal karyotype at an endogenous locus by a genetic targeting event...". As established in the attached Declaration of David L. Ayares, Ph.D., Dr. Ayares states that he "knows of no somatic cell that cannot be cloned as long as it has a normal karyotype". Dr. Ayares explains that the general technique of nuclear transfer is well known, and that when using a new donor cell type for somatic cell nuclear transfer, the worker simply has to recognize that it is a numbers game, and one must repeat the experiment using the well known techniques until success is achieved. Dr. Ayares establishes that it can routinely require hundreds or thousands of embryo transfers to produce a viable cloned animal, which can take as little as two weeks to perform on a routine basis.

The Oback paper reports false negative results in presenting data that some somatic cells have a 0% cloning efficiency. Table 1 of Oback reports the cloning efficiency of fibroblast cells, which have been the most commonly used donor cell type for nuclear transfer. Oback reports a cloning efficiency for fibroblasts of between 0.05% and 1.2%. A cloning efficiency of 0.05% represents 1 viable clone (i.e. live birth) per 2000 embryos transferred. However, as established in Dr. Ayares declaration, when Oback presented the data in Table 1, the actual number of embryos transferred was not included. Dr. Ayares reviewed the primary references from which Oback compiled Table 1 to determine the actual number of embryos that were transferred for each cell type that Oback reported the cloning efficiency to be zero. In fact, as seen in the attached declaration, only 0-75 embryos were actually transferred in those experiments which failed to produce viable cloned offspring. As evidenced by Oback's own cloning efficiency data for the most commonly cloned cell type, fibroblasts, cloning efficiencies requiring 2000 embryo transfers are sometimes necessary even for this well-established somatic donor cell type. In fact, of the 5 somatic cell types reported by Oback to have a 0% cloning efficiency (mature sertoli cells from the testis, lymphocytes from the thymus, macrophages from the peritoneal cavity, leukocytes from the spleen and neuron/glia from the brain), researchers have since cloned animals using neurons, lymphocytes and leukocytes as somatic donor cells (Yamazaki, et al, *Proceedings of the National Academy of Sciences*, November 20, 2001, Vol 98, pages 14022-14026; Hochedlinger & Jaenisch *Nature* 2002 Feb 28 415(6875):1035-8. Epub 2002 Feb 10).

Further, to date, at least thirty somatic donor cells have been used to date to clone animals (see, "Somatic Cell Nuclear Transfer Cloning Efficiency" Paterson & Wilmut: www.roslin.ac.uk/public/webtablesGR.pdf).

(ii) Homologous Recombination

The Examiner also rejected Claims 62-67, 70-73, 75-90, 98-127 and 131-133 as failing to comply with the enablement requirement on the basis that the state of the art of homologous recombination was unpredictable at the filing date and, as discussed during the interview with the Examiners, can only be used with cells that have high proliferative potential.

Homologous recombination is a natural event that occurs in all cells, and in fact is required for cell viability. In nature, homologous recombination underlies many biological pathways. The recombination machinery has been well conserved throughout evolution as an essential component of cell survival. Homologous recombination is the leading technology in rational genome engineering. Scientists have exploited this fundamental cellular process to manipulate genomes. Applicants do not need to establish that any specific cell can undergo homologous recombination because, in fact, all cells can and do undergo homologous recombination.

During the interview with the Examiners, the issue was raised regarding whether cells other than fibroblasts could be targeted since other cell types may not have equivalent proliferative potential. As discussed with the Examiners, it was clearly feasible as of the filing date of the present application to obtain and screen for homologous recombination in cell types that do not have high proliferative potential. As established in Dr. Ayares declaration, as early as 1990, several methods, including PCR-based and FACS-based techniques, were known to those skilled in the art, which could be used to detect targeted integration events after homologous recombination within 3-5 days (Zimmer and Gruss 1989 Nature 338: 150-153; Jasin et al 1990 Genes & Development 4: 132-166). These methods allowed one to identify targeted clones, in a variety of cell types, without prolonged in vitro growth and expansion. It was not a pre-requisite as of the filing date that cells to be used for homologous recombination and subsequent nuclear transfer must have high or even moderate proliferative potential.

(iii) Recipient Cells

The Examiner further rejected Claims 62-67, 70-73, 75-90, 98-127 and 131-133 as failing to comply with the enablement requirement on the basis that the claims are overbroad with respect to recipient cells. The Examiner indicates that the breadth of the claims recite methods of nuclear transfer utilizing recipient cells, which include, but are not limited to oocytes. The Examiner indicates that besides oocytes, other types of recipient cells can be used, such as zygotes and two cell embryos. Applicants have amended the claims from "transferring the modified nuclear genome of the somatic cell to a recipient cell to produce a nuclear transfer unit" to recite "transferring the modified nuclear genome of the somatic cell to an oocyte, two cell embryo or zygote to produce a nuclear transfer unit".

(iv) Species/ Genus

With regard to Applicants' reference in claims 62, 90 and 131-133 to the transfer of the embryo to a surrogate mother, the Examiner has requested that the Applicants clarify on the record that they are referring to the gestation of an animal in the womb of an animal of the same species. Applicants hereby clarify that the claims are limited to transferring the embryo to a surrogate mother that is able to carry the embryo to term. Therefore, the term "species", as referred to in the Applicants' correspondence, is meant to describe those animals within a taxonomic genus.

(v) Abundant Expression

The Examiner further rejected the pending claims as failing to comply with the enablement requirement on the basis that the term "abundant expression" is overbroad. Applicants have amended claims 73 and 105, which previously recited the term "abundant or abundant expression" to no longer include those terms. Claim 101 has been canceled without prejudice.

(vi) Genotype/ Phenotype

The Examiner further rejected claims 62-67, 70-73, 75-90, 98-127 and 131-133 as failing to comply with the enablement requirement on the basis that the claims are overbroad with respect to producing any non-human animal whose genome contains a modification at an endogenous locus by a genetic targeting event. Specifically, the Examiner asserts that the claims

are not enabled because genetically modified animals without a distinct phenotype would not be discernable from wild-type animals.

The modification of a genome of an animal by means of a genetic targeting event creates an animal that allows the expression of a desired protein or the disruption of a gene expressing an undesired protein. As the Examiner is aware, not all genes are ubiquitously expressed in all cell types. There are many proteins that are turned on or off as a result of a signaling mechanism. Therefore, transgenic animals will not always display the genetic changes, but may only reveal the modifications upon expression or lack of expression of the targeted gene. Further, as the Examiner is well aware, scientists can identify animals with modified genomes using routine techniques, such as PCR.

Claim Rejections - 35 U.S.C §112, 2nd Paragraph

The Examiner has rejected Claims 90, 97-127 and 133 as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 90 and 133 were rejected for lack of antecedent basis for "the nuclear transfer unit" in step (c). Applicants have amended claims 90 and 133 so that antecedent basis is now provided in step (b). The term "abundant" in claim 105 was rejected for indefiniteness. The term "abundant" is no longer recited in claim 105. Claim 133 has also been amended such that it no longer reads "in a manner that accomplishes breeding".

It is respectfully submitted that this application is now in condition for allowance. If the Examiner considers, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Enclosed is a check for $905.00 ($510.00 for the three-month extension of time fee and $395.00 for the Request for Continued Examination (RCE) fee). No additional fees are believed to be due. However, should the Commissioner determine otherwise, he is authorized to charge any such fee or to credit any overpayment to Deposit Account No. 11-0980.

Respectfully submitted,

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Date: July 25, 2005